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Validation of a molecular screening tool for the detection of chromosomal abnormalities in donkey

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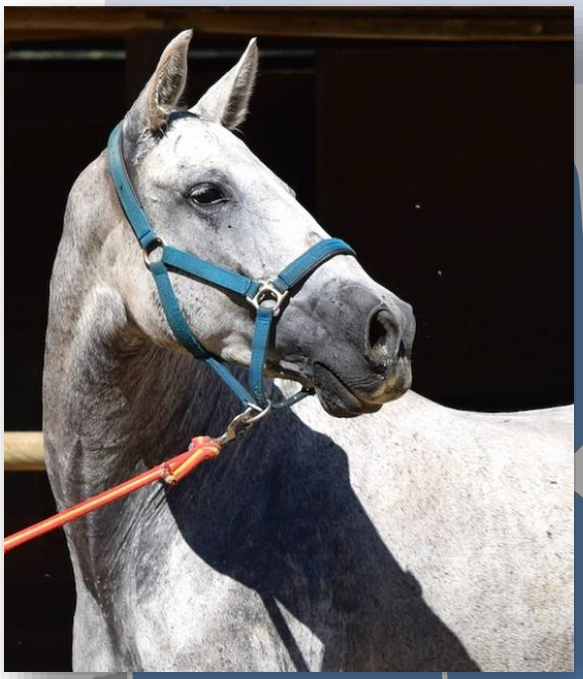
INTRODUCTION

Horse



vs.

Donkey

sexual chromosomes

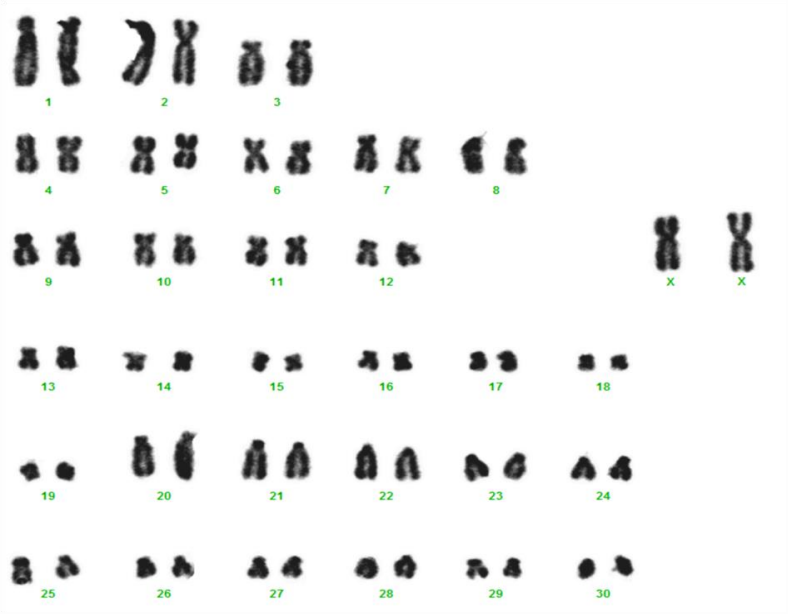
INTRODUCTION

Chromosomal alterations in equines

- Congenital anomalies
- Embryonic losses
- Infertility

Production

Economic and time loss

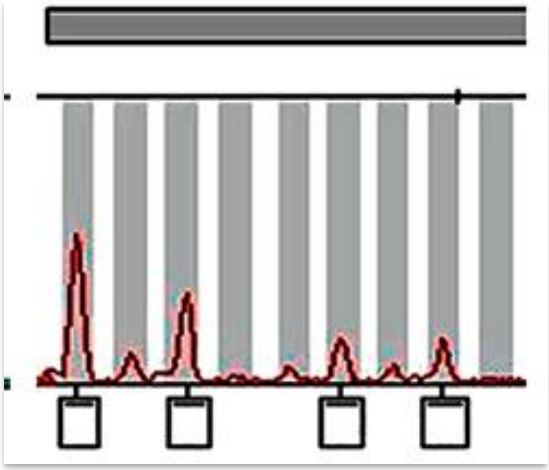


Validation of a molecular screening tool for the detection of chromosomal abnormalities in donkey

OBJECTIVE OF THIS WORK

To analyse the possibility of **applying** an **STR** (Single-Tandem-Repeat)-based **molecular method** developed **for horses** as a diagnostic tool for sexual chromosomes abnormalities **in donkeys**.

XX - XY



MATERIALS AND METHODS

Animals: 121 donkeys (51 Andaluza breed and 70 Moruna)
93 females and 28 males

Hair

Cellular cultures

DNA isolation:

Molecular analyses

MATERIALS AND METHODS

Marker	Localization	Type
LEX003	X	Microsatellite
LEX026	X	Microsatellite
TKY270	X	Microsatellite
TKY38	X	Microsatellite
UCEDQ502	X	Microsatellite
ECAYM2	Y	Microsatellite
SRY	Y	Gene

DNA amplification

Multiplex PCR

PCR products genotyped by capillary electrophoresis

Alelle sizing

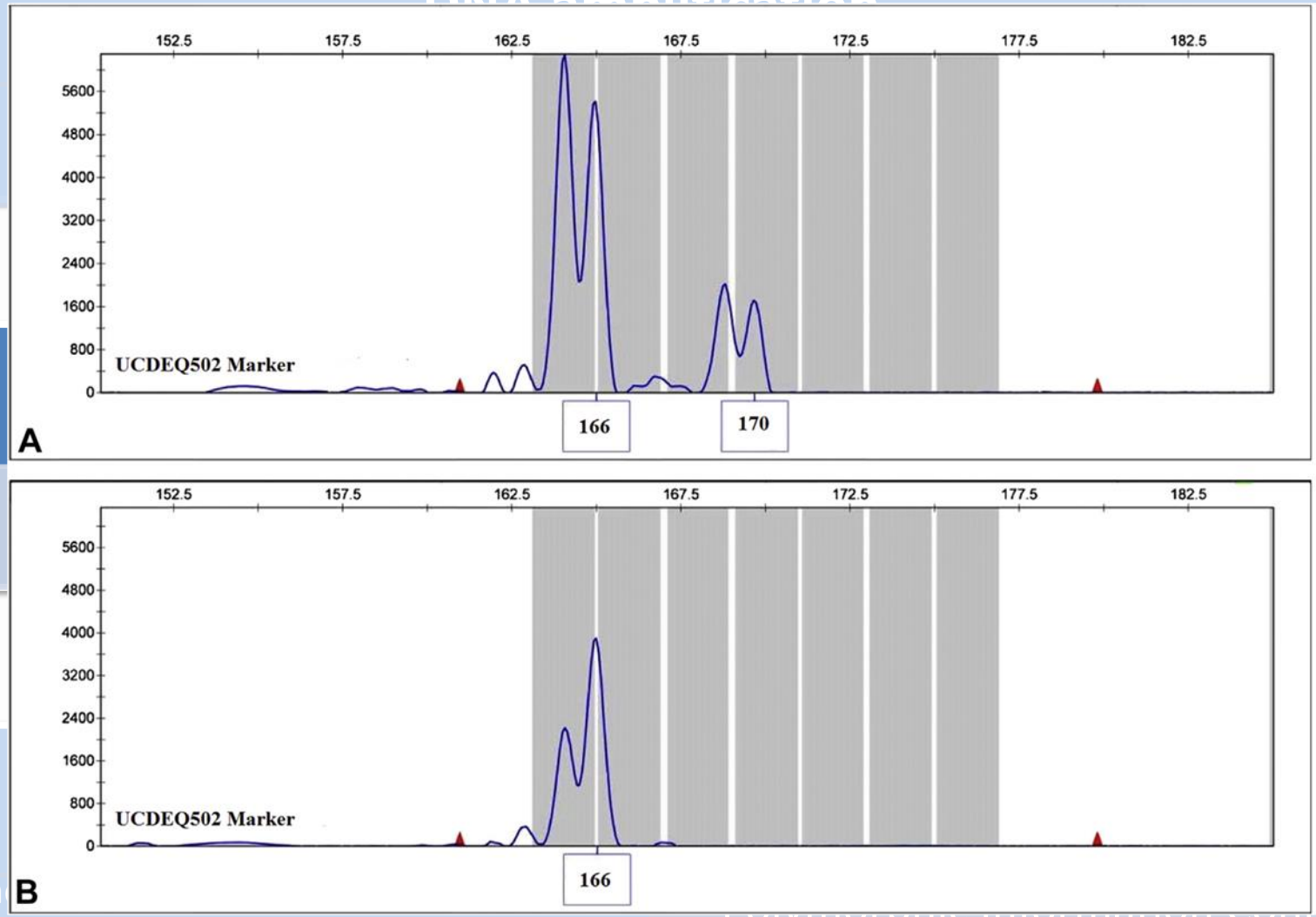
GeneMapper © 4.0 software

Data analysis

Frequency, average and total number of alleles. Observed and expected heterozigosity. Genetix 4.05.2 software

Polymorphic Information Content (PIC). Cervus 3.0.7. software

MATERIALS AND METHODS



Observed and expected heterozygosity. Genetix 4.05.2 software

(PIC). Cervus 3.0.7. software

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Polymorphic Information Content (PIC). Cervus 3.0.7. software

RESULTS

- No differences between DNA from blood and hair.

- Y chromosome gene and microsatellite (*SRY* and *ECAYM2*): detected in 100% of the animals phenotypically described as males and 0% of females.

STR	Number of alleles	Average number of alleles	Total number of alleles
LEX003	9		
LEX026	5		
TKY270	8	9.6	48
TKY38	12		
UCEDQ502	14		

RESULTS

Genetic characterisation of the population

STR	H_{obs}	H_{ex}	PIC
LEX003	0.3118	0.6126	0.5617
LEX026	0.3696	0.6509	0.6149
TKY270	0.3978	0.5478	0.4636
TKY38	0.3516	0.6434	0.6074
UCEDQ502	0.4409	0.8573	0.8513

Polymorphic Information Content (PIC) > 0.5 → High informative quality of this set of molecular markers

RESULTS

Sensitivity and specificity

Phenotype	Diagnosis	Karyotype	X markers profile	Y markers	
				ECAYM2	*SRY*
Male	Normal male	62, XY	One allele per marker	+	+
Female	Normal female	62, XX	At least one marker in heterozygosis	-	-
Female	Complete Turner'ssyndrome	61,X0	One allele per marker	-	-
Male/Female	Cellular chimerism	62, XX/XY	At least one marker with more than 2 alleles Possible hair/blood differences	+	+
Male	Male SRY positive/negative Disorder in Sex Development	62, XX	At least one marker in heterozygosis	-	+/-
Female	Female SRY positive/negative Disorder in Sex Development	62, XY	One allele per marker	+	+/-

CONCLUSIONS

This molecular tool could be used as a diagnostic technique for the detection of chromosomal anomalies of the sexual pair in donkeys.

Fast

Reliable

High sensitivity

Cheap

High specificity